I. CARDIOVASCULAR, PHARMACOLOGY and CHEMISTRY
II. EPIDEMIOLOGY, STATISTICS and PSYCHOSOMATIC

NO. 629

THE COUNCIL FOR TOBACCO RESEARCH - U.S.A

633 THIRD AVENUE
NEW YORK, N. Y. 10017

COMMITTEE:
Dr. Cattell, Chm.
Dr. Little
Dr. Jacobson

Application For Research Grant

Approved

Date: August 7, 1967

1. Name of Investigator(s): (include Title and Degrees)

Carlton K. Erickson, Assistant Professor of Pharmacology and Toxicology, B.S., M.S., Ph.D.

2. Institution & Address:

School of Pharmacy
The University of Kansas
Lawrence, Kansas 66044

3. Short Title of Project:

Mechanism of Learning Facilitation by Nicotine

4. Proposed Starting Date:

November 1, 1967

5. Anticipated Duration of this Specific Study:

12 months

6. Brief Descripton of Objectives or Specific Aims:

For obvious social and medical reasons, nicotine is one of the common drugs which has been studied for its effect on learning and performance. In humans who smoke regularly and inhale a significant amount of nicotine, obvious questions arise concerning the effects of prolonged nicotine absorption on the subject's alertness and learning ability. Obviously, nicotine would be considered socially unacceptable if it seriously impaired alertness and learning. Recent research, however, has demonstrated that while large doses of nicotine depress learning, small doses can enhance performance in rats and mice (1,3). Unfortunately, there has not been a concerted effort to study the true mechanism of nicotine's observed facilitation of learning. McGaugh and Petrinovich (9) have cautioned against assuming that changes in performance reflect actual changes in efficiency of the neural processes involved in learning (i.e., the so-called formation of the memory trace). It is of special practical importance with nicotine to differentiate a transient change in motivation or alertness from the relatively permanent alteration of neural processes involved in learning.

Experience has shown that in order to convincingly demonstrate that nicotine (or any centrally-active drug) is actually affecting neural learning mechanisms, 3 important criteria must be fulfilled:

1. The phenomenon of "drug dissociation" must be ruled out. McGaugh and Petrinovich (9) have stated that "subsequent studies of nicotine (on learning) should take into account the possibility that dissociation may occur with this compound". Drug dissociation refers to the situation in which habits learned by subjects in a drugged state do not transfer to the normal state, but can be evoked again whenever the subject is drugged. In other words, nicotine may act as a secondary stimulus during learning.

(to page 1a)

7. Give a Brief Statement of your Working Hypothesis:

Nicotine enhances central nervous processes involved in learning and does not demonstrate deleterious effects on behavioral performance in doses comparable to those involved in smoking.

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Objectives and Specific Aims (continued):

of a response or other information; when the drug is not present, the learned material may not be recalled.

2. Peripheral actions of the drug must be ruled out. It is well known that changes in peripheral functioning such as autonomic and ganglionic transmission changes, sight and hearing changes, etc. can influence learning. These must be shown to be inoperative in any suspected drug effects on the learning process.

3. The contribution of central "side effects" must be determined. In order that the effect of a centrally-active drug be pinpointed to its action on central learning processes, other central effects such as changes in motivation (hunger, thirst) and excitability level (anxiety, confusion, hyperesthesia) must be ruled out. Contribution of these effects to observed performance would not be considered part of true learning alteration since they are temporary and do not directly influence permanent memory.

This project, then, is designed to fulfill the preceding criteria for nicotine by a series of appropriate pharmacologic and neurophysiologic experiments. Since the hippocampus, a limbic system structure, has been implicated by many workers as a major brain area involved in learning, this as well as other suspected structures (cortex, reticular activating system) will be concentrated upon. It is interesting that low doses of nicotine which enhance learned performance in animals also produce a characteristic theta rhythm (4-7 cps slow wave activity) in the hippocampal EEG (8). Conversely, high doses of nicotine which block learned behavior can elicit seizure activity in the hippocampus. Although these observations have never been correlated in the same animal, they provide an interesting theoretical basis for studying the relations between nicotine, learning, and the hippocampus and other related brain structures.

A unique technique to be used in this study concerns the "chronic" administration of small doses of nicotine during a relatively lengthy avoidance training session. Because of the short duration of action of nicotine, rats will be fitted with chronically-indwelling intravenous catheters for repeated nicotine administration. It is felt that this will also closely parallel the effects of a chain smoker performing a relatively complex mental or physical task.

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8. Details of Experimental Design and Procedures: (Attach Separate Pages)

Animals - Rats will be used throughout the study, in order to satisfy current housing requirements and to allow data accumulation on a large number of inexpensive subjects.

Training procedure - Since most of the published behavioral studies with rats and nicotine have utilized either the shuttlebox or pole-climb avoidance response, it seems best to expand upon these results with a similar behavioral situation. This worker is most familiar with the discriminated lever-press avoidance response and its interpretations, so this training situation will be used. The procedure is described in detail in an accompanying reprint (Psychonom. Sci., 1967). The procedure is especially advantageous for learning studies because acquisition of the response is relatively slow, thereby allowing accurate observation of changes in response rate due to drug treatment.

Drug administration method - Since the purpose of this project is to study the effects of nicotine on learning rather than on previously-learned behavior, nicotine and saline (control) will be administered during training sessions only. That is, doses will be administered up to the time when a preset criterion is reached (either 85% avoidance average for 3 consecutive sessions, or 15 sessions, whichever is reached first). Although doses, onsets, and durations will have to be determined for our particular test conditions and animals, the literature does provide

9. Physical Facilities Available (Where Other than Administering Organization Indicate Geographical Location) (to page 2a)

The applicant has at his disposal for this project the following facilities and equipment:

1-Staff office (air conditioned)
1-Laboratory-office combination containing desks for 5 students and animal surgery area (air conditioned) (to page 2c)

10. Additional Requirements:

For completion of programming equipment in available test chambers:

2-Grason Stadler shocker-scramblers, Model E1064GS
1-Heavy-duty power supply, 28VDC (Grason Stadler), Model E783DA
2-Lehigh Valley basic interval timer, Model 1309
1-Lehigh Valley session timer, Model 1350C
1-Lehigh Valley digital counters, Model 1425-10

11. Biographical sketches of all principal and professional personnel (append)

See page 2d.

12. List of publications: (Five most recent as pertinent) (append)

See page 2e.

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Experimental Design and Procedures (continued):

information which can be used to design the dose schedule. Domino (3) has shown that nicotine in relatively small doses reaches its peak after 5 minutes and has a duration of approximately 30 minutes. Since the avoidance training method to be used requires 4-hour sessions every other day, it is obvious that nicotine will have to be given more than once in order to provide prolonged drug action for an entire session. Thus animals will be fitted with chronic-indwelling catheters via the jugular vein according to a method described by Weeks and Davis (11) and modified by us (5). The cannula will be attached to a swivel fluid-lock assembly mounted in the top of the test chamber which will allow the animal to move freely without twisting the cannula. Injections will then be made at desired intervals without physically interrupting the animal's training session.

Doses to be used - According to Johnston (6), 1/50 gr. of intravenous nicotine produces, in a habitual smoker, the same psychic effects as smoking one cigarette. Assuming this effect occurs in a 70 kg. man, the "cigarette equivalent" of nicotine received is calculated to be 18.6 ug/kg. of body weight. Since our experimental design will utilize intravenous nicotine administration, the nicotine "cigarette equivalent", as well as other similar small doses, will be thoroughly investigated. Along these lines, it is interesting that the doses used by Domino (3) to facilitate avoidance behavior (40-80 ug/kg. subcutaneously) and by Brown (2) to evoke hippocampal responses (30-50 ug/kg. subcutaneously) are similar to our calculated cigarette equivalent. It further seems very possible that our 18.6 ug/kg. dose given intravenously may produce the same effects as the Domino and Brown subcutaneous doses.

Experimental plan - The 3 criteria discussed under Objectives will be investigated using nicotine as follows:

1. Drug dissociation - In order to determine the presence or absence of nicotine dissociation of learning, groups of rats will be trained in the conditioned avoidance situation under the effects of nicotine and saline. Rate of response acquisition to the preset criterion will be recorded to determine enhancement of response rate with nicotine. Later sessions will consist of performance measurement of both groups treated with saline. Drug dissociation will be ruled out if the nicotine-trained rats continue to perform at the pre-saline level.

2. Central action of nicotine - The usual method of determining whether a drug is acting centrally is to demonstrate a behavioral action with the drug, followed by loss of the action with a quaternary derivative which will not pass the blood-brain barrier. In this regard, Domino (4) has reported that congeners such as nicotine methiodide are relatively ineffective in contrast to nicotine in blocking conditioned avoidance behavior in rats. Nicotine methiodide will be used to confirm these results in our conditions, and to control for central specificity in other parts of the study.

Another method to test for specificity of drug effects in the brain is simply to place the drug into various structures of interest by means of a cannula. This provides a means of determining significant differences in drug effect from parenteral administration. Using apparatus similar to that described above for intravenous injections, rats with chronically-indwelling cannulas in the brain will be tested with small doses of nicotine injected into the cortex, hippocampus, and reticular formation. Intracerebral quantities of nicotine will approximate 1×10^{-9} moles, and solution volumes will be not more than 2 uL/injection. A modification of the nylon-stainless steel assemblies of Valenstein et al. (10) will be used for cannula construction, and nicotine will be administered intracerebrally at times comparable to parenteral administration.

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Confirmation of cannula placements will be performed histologically. Frozen sections of 50 micron thickness will be cut, placed on slides, and stained with cresylechtviolett and methylene blue according to the method of Kluver and Barrera (7).

3. Direct action on neural learning processes - The procedure of administering drugs after training rather than before training has been adopted by a number of investigators as a means of assessing memory storage effects by ruling out motivational changes and excitability factors. A current popular theory of learning and memory states that stimuli entering the CNS initiate reverberating neural pathways which must continue for a fixed time to form a memory trace. The longer the pathway reverberates and the stronger the incoming stimulus, the more deeply the memory trace is implanted. Thus any overriding stimulus (e.g., electroshock or certain drugs) which disrupts the reverberating neural pathway (assuming the pathway is electrical-chemical in nature) will inhibit learning and trace formation. Post-trial electroshock and various drugs such as barbiturates and tranquilizers have indeed been shown to affect the learning process in this manner (9). On the other hand, central stimulants such as emphetamine and strychnine given after training trials in animals have been shown to enhance the learning process (9). This technique is especially suitable for drugs which are maximally effective within minutes and which are metabolized prior to retention tests, as is nicotine. Thus, enhancement of conditioned avoidance learning when nicotine is given after each session would strongly indicate facilitation of memory trace formation by this drug.

References

1. Bovet, D., Bovet-Nitti, F., and Oliverio, A., "Effects of nicotine on avoidance conditioning of inbred strains of mice", Psychopharm. 10: 1-5 (1966).

2. Brown, B.B., "Relationship between evoked response changes and behavior following small doses of nicotine", Ann. N.Y. Acad. Sci. 142:190-200 (1967).

3. Domino, E.F., "Some behavioral actions of nicotine", in Tobacco Alkaloids and Related Compounds, ed. Von Euler, The MacMillan Company, New York (1965), pp. 145-62.

4. Domino, E.F., "Some comparative pharmacological actions of (-)-nicotine, its optical isomer, and related compounds", in Tobacco Alkaloids and Related Compounds, ed. Von Euler, The MacMillan Company, New York (1965), pp. 303-13.

5. Erickson, C.K., unpublished data on chronic intravenous alcohol infusion in rats.

6. Johnston, L.M., "Tobacco smoking and nicotine", Lancet 243:742 (1942).

7. Kluver, H. and Barrera, E.A., "A method for the combined study of cells and fibers in the nervous system", J. Neuropathol. exp. Neurol. 12: 400-3 (1953).

8. Longo, V.G., Giunta, F., and Scotti de Carolis, A., "Effects of nicotine on the electroencephalogram of the rabbit", N.Y. Acad. Sci. 142:159-69 (1967).

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Source: https://www.industrydocuments.ucsf.edu/docs/mxwm0000

9. McGaugh, J.L. and Petrinovich, L.F., "Effects of drugs on learning and memory", Intern. Rev. Neurobiol. 8:139-96 (1965).

10. Valenstein, E.S., Hodos, W., and Stein, L., "A simplified electrode-assembly for implanting chronic electrodes in the brains of small animals", Am. J. Psychol. 74:125-8 (1961).

11. Weeks, J.R. and Davis, J.D., "Chronic intravenous cannulas for rats", J. Appl. Physiol. 19:540-1 (1964).

Physical Facilities Available (continued):

1-Behavioral laboratory (air conditioned) containing 9 rat test chambers and relay programming equipment (3 test chambers will be available for this project; some programming equipment is required to complete the chambers - see Additional Requirements)
1-Histological laboratory (for confirmation of cannula placement work), with microtome equipped for carbon dioxide and paraffin sectioning

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Recent Publications:

Chalmers, R.K. and Erickson, C.K., "Central cholinergic blockade of the conditioned avoidance response in rats", Psychopharmacologia 6:31-41 (1964).

*Erickson, C.K. and Chalmers, R.K., "Hippocampal theta rhythm involvement in cholinergic-induced blockade of discriminated avoidance responding in rats", Arch. int. Pharmacodyn. 163:70-8 (1966).

*Erickson, C.K., "Facilitated responding in a discriminated lever press avoidance situation", Psychonom. Sci. 8:37-8 (1967).

Erickson, C.K., "A reliable lever-press avoidance training method in the rat", Fed. Proc. 26:738 (1967).

Patel, J. and Erickson, C.K., "Enhanced avoidance acquisition by low-intensity hippocampal stimuli", Pharmacologist 9:200 (1967).

* Reprint accompanies proposal

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13. Budget: (1st year)

A. Salaries (Personnel by names)	% time	Amount
Professional		
Carlton K. Erickson, Prin. Invest.		
9 months academic year	20%	Not requested
3 months summer	100%	R
Fringe benefits		
Technical		
Jitendra B. Patel, Graduate Student		
12 months calendar year	50%	$ R
Sub-Total		$ R
B. Consumable Supplies (list by categories)		
Animals and feed		$ 400
Cannula materials		100
Drugs and histological chemicals		100
Recorder paper, notebooks		50
Sub-Total		$ 650
C. Other Expenses (itemize)		($ 7,075)
None		
Sub-Total		
D. Permanent Equipment (itemize)		
2-Grason Stadler Shockers with Grid Scrambler, Model E1064GS @ $ 285.00		$ 570
1-Grason Stadler Heavy-duty Power Supply, 28VDC, Model E783DA		175
2-Lehigh Valley Basic Interval Timers, Model 1309 @ $ 95.00		190
(to page 3a) ... Total-		$ 1,214
E. Overhead (16% of A+B+C)		$ 1,061
Total		$ 9,350

Estimated Future Requirements: NONE

	Salaries	Consumable Suppl	Other Expenses	Permanent Equip.	Overhead	Total
Year 2						
Year 3						

It is understood that the applicant and institutional officers in applying for a grant have read and found acceptable the Council's "Statement of Policy Containing Conditions and Terms Under Which Project Grants Are Made."

Signature Carlton K. Erickson
Director of Project
913 UN4-4004
Telephone

Signature Raymond Nichols
Business Officer of the Institution
VICE CHANCELLOR FOR FINANCE
UN-3136
Telephone

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D. Permanent Equipment (continued)

1-Lehigh Valley Session Timer, Model 1350C	$ 80
1-Lehigh Valley Digital Counters, Model 1425-10	$199

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Other Sources of Financial Support

List financial support for research from all sources, including own institution, for this and/or related research projects.

Current

Title of Project	Source	Amount	Duration
"Addictive Ability of Self-injected Intravenous Alcohol in Rats"	Licensed Beverage Industries	$ 7,965	11/1/66 - 10/31/67
"Behavioral Hyperexcitability Induced by Alcohol Withdrawal in Rats"	University of Kansas Research Committee	$ 3,000	7/1/67 - 6/30/68

Pending

"Cholinergic Implications of Ethanol Intoxication"	National Institute of Mental Health	$ 11,925 ($ 35,700)	1/1/68 - 12/31/68 (1/1/68 - 12/31/70)
"Enhancement of Learning by Limbic System Stimulation"	National Institute of Mental Health	$ 25,118 ($ 66,586)	1/1/68 - 12/31/68 (1/1/68 - 12/31/70)

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